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EFFECTS OF VASCULAR PERFUSION ON THE ACCUMULATION, DISTRIBUTION AND TRANSFER OF 3-*O*-METHYL-D-GLUCOSE WITHIN AND ACROSS THE SMALL INTESTINE

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SUMMARY

1. Factors affecting the transfer of the non-metabolized, 'actively transported' sugar, 3-*O*-methyl-D-glucose (3MG) across the small intestinal epithelium have been examined in vascularly perfused anuran intestine. Transfer has been studied during absorption in the steady state, and also during the period of transition from one steady state to another.

2. During the steady state, the rate of absorption of 3MG from the intestinal lumen is equal to the rate of appearance in the portal venous effluent; this rate of transfer is to a small, but significant, extent directly related to the rate of arterial perfusion. With phlorizin in the intestinal lumen, transfer across the epithelium is reduced to very low rates which are independent of the rate of vascular perfusion.

3. The apparent size of the tissue pool(s) of 3MG that have to be loaded to achieve the steady state rate of transfer are less than those that unload into the vascular bed after 3MG is removed from the intestinal lumen. This 'up–down asymmetry' is abolished when phlorizin is present in the intestinal lumen during the unloading phase.

4. When 3MG is abruptly removed from the intestinal lumen after the tissue has been previously loaded with the sugar, the rate of washout into the vascular bed can be described by the sum of two exponential terms. The two terms differ in that the rate constant of the earlier 'fast' term is sensitive to the rate of vascular perfusion, while the later, 'slow', rate constant is insensitive to flow rate. The total quantity of 3MG that can be unloaded from the tissue into the portal venous effluent is decreased when phlorizin is present in the intestinal lumen during the unloading phase.

5. Absorption from the lumen of the anuran intestine continues while the mesenteric circulation is interrupted. An estimate of the concentration of 3MG during the period of vascular stoppage can be made from the quantity recovered in the portal venous effluent when vascular perfusion is reinstituted ('vascular stop-flow'). The extent of the accumulation depends upon the duration of the vascular stoppage and the presence of Na ions in the intestinal lumen is essential for accumulation to occur.

6. The findings are discussed in relation to the transfer of 3MG between various

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possible compartments in the tissue during absorption. Evidence is presented that a re-uptake of previously absorbed 3MG may occur across the brush border membrane. Such a recycling of 3MG across the epithelium implies that the apparent unidirectional fluxes measured across the epithelium between the bulk phase of the lumen and the blood may underestimate the size of fluxes across the epithelium at the cellular level.

INTRODUCTION

In vitro methods have thrown much light on processes underlying intestinal absorption, but a characteristic of such preparations is that the composition of the fluid environment adjacent to the transluminal surface of the epithelium is determined almost entirely by the effects of epithelial transport. Furthermore, in the absence of a blood supply the composition of this environment is unlikely to be the same as that *in vivo* and will not remain constant during *in vitro* experiments. By perfusion of the vascular bed the composition of this environment can be controlled (see, for example, Parsons & Prichard, 1968). Here we attempt to examine the influence of vascular flow on the epithelial transport of the non-metabolized glucose analogue 3-*O*-methyl-D-glucose (3MG) by taking advantage of the fact that in the vascularly perfused preparation of anuran intestine already described (Boyd, Cheeseman & Parsons, 1975*a*) it is possible to interrupt vascular flow for varying periods of time. By turning a switch the preparation can be converted from the equivalent of an *in vivo* preparation to an *in vitro* one and back again. The changes occurring within the intestinal wall during a period of vascular stop may be discovered, at least in part, by examining the recovery of substances in the vascular effluent immediately following the reinstitution of vascular flow in a fashion that is analogous to stop flow experiments used to study the kidney (Malvin & Wilde, 1973).

Preliminary accounts of some of the work described here have been given (Parsons, 1975; Boyd, 1977; Boyd & Parsons, 1976, 1977).

METHODS

The vascularly perfused preparation of frog small intestine has been described previously (Boyd *et al.* 1975*a*). The salient features are that fluid flows simultaneously through both the lumen and the vascular bed by single pass. The effluents flowing from the lumen and from the portal vein are recovered quantitatively at appropriate intervals by means of two fraction collectors. We have used two methods to study the properties of the perfused epithelium. In the 'perturbation' method, the intestine is loaded from the lumen with the substance(s) whose transport is being studied ('substrate(s)') and the washout into the vascular bed is then studied. The 'vascular stop flow' method involves the intermittent vascular perfusion of the preparation; upon resumption of flow attention is paid to the washout of substrate trapped in the intestine during the period of vascular stoppage.

Perturbation method

A rate of flow of luminal fluid sufficient to allow both the abrupt introduction of substrate into and its abrupt removal from the lumen is necessary for this procedure. This rate was established using a 'dummy' intestinal lumen made from rubber tubing. It was found that by using luminal flow rate greater than 2 ml. min^{-1} the 'bulk phase' of the intestinal lumen may be filled and emptied relatively abruptly, with linear washout kinetics with rate constant of the order of 0·5 min^{-1}. On occasion short segments (some 5 cm in length) of intestine were

employed. Use was made of inhibitors of transport, such as phlorizin, to produce pharmacological washout experiments by blocking transport (see Results).

Routinely the appropriate substrates were introduced into the lumen as a loading 'pulse' delivered from a preloaded circuit (20 or 50 ml. capacity) arranged in parallel with the input to the intestinal lumen and preloaded with Ringer of the desired composition. The contents of this circuit could then be delivered into the lumen by turning two three-way taps. The advantages of this system are, first, the substrate-containing solution may be introduced at a rate identical to that of the substrate-free solution; secondly, the sharpness of the profile of the pulse is markedly improved by introducing the substrate from the circuit for a precisely timed period and the exact quantity delivered to the lumen may be calculated; thirdly, the circuit can be reloaded allowing the repetition of the pulse with or without alteration of some further variable.

The size of the 'loading pool', the quantity of substrate required to be present in the tissue in order to achieve a constant rate of transfer into the vascular effluent, was calculated using the measured delay in the time taken to reach the steady state following the addition of substrate to the lumen (Andersen & Zerahn, 1963). The 'unloading pool' size was derived from the quantity of substrate that appears in the vascular effluent following the removal of substrate from the lumen. Provision was made for the dead space (0·58 ml.) of the portal cannula in both these calculations (see Boyd *et al.* 1975*a*).

Simultaneous estimation of unidirectional fluxes across the epithelium

3MG was added at the same concentration to both the luminal and vascular perfusate, the former containing ^{3}H-labelled and the latter ^{14}C-labelled isotopes of the sugar. The rates of appearance of the respective isotopes in the 'trans' compartment were measured in the routine way, and from this the unidirectional fluxes of the monosaccharide across the epithelium calculated.

Vascular stop flow

Following the institution of the vascularly perfused preparation, substrate-containing fluid is pumped through the lumen until a steady rate of transfer across the epithelium is achieved. The vascular pump is then either turned off or set at minimum setting (about 1 % of the vascular flow rate) for a period of 30 min. The switch to minimum setting is technically preferable to a complete switch off. It is probable that the epithelium remains unperfused at this rate of pumping. The term vascular stop flow is used to describe both situations. Samples are taken at short time intervals following resumption of flow for accurate determination of washout kinetics. Steady state appearance of substrate, determined previously with both pumps operating, is subtracted from that seen following temporary cessation of vascular perfusion to obtain washout profiles. The conditions obtaining in the lumen are kept constant throughout the entire vascular stop flow.

It may be calculated from estimates of the Q_{O_2} and the maximal rate of glucose utilization by frog intestine observed by Prichard (1966) that during the periods of 'vascular stop' sufficient oxygen is supplied to the epithelium from the fluid continually flowing through the lumen. The finding that transport may be maintained at a steady rate during 'vascular stop' and that complete return to the steady rate of transport is achieved upon resumption of vascular flow indicate that the procedure of 'vascular stop flow' does not produce irreversible changes in the systems for epithelial transport.

Measurement of absorption of 3MG from the lumen

This was performed by use of a non-absorbed extracellular marker. ^{14}C-labelled sucrose was routinely used (the frog small intestine lacks sucrase). This has been shown previously to be an appropriate marker (Boyd, Cheeseman & Parsons, 1975*b*). The rate of 3MG absorption was derived from measurement of the concentration of marker and monosaccharide in the fluid introduced into the intestinal lumen (I_M and I_s respectively) and in the fluid, collected in timed fractions, flowing out of the lumen (O_M and O_s respectively). At luminal flow rate, F, the rate of 3MG absorption from the lumen, A, in each fraction of luminal effluent is given by

$$A = \left[\left\{O_M \cdot \frac{I_s}{I_M}\right\} - O_s\right] F.$$

General conduct of the experiments

Two species of frog, *Rana ridibunda* (Mavad, Budapest) and *R. pipiens* (Carolina Tips, U.S.A.) were used. The former was found to transport sugars at a markedly faster rate and was used routinely. Both species were maintained as previously described (Boyd *et al.* 1975*a*) and were fed twice weekly on living house-crickets (Xenopus Ltd., Redhill, Surrey).

Ringer fluids for the vascular and luminal circuits were those previously described (Boyd *et al.* 1975*a*). In Na-free experiments, K or choline was used as the substituent cation. The calculation of rates of transfer, of washout rate constants and of loading and unloading pool sizes was as previously described.

Control and experimental periods were conducted on the same intestine. These periods could not always be randomized because certain procedures were more easily performed at the start (e.g. Na-free conditions in the lumen) or at the end (e.g. addition of phlorizin to the lumen) of an experimental sequence. Nevertheless reproducibility and some degree of reversibility was shown for all the phenomena here described. Under standard conditions and within a single batch of animals it should be noted that the absolute rates of transfer varied in different animals; the variations found between different batches could be larger.

Analytical methods

The rates of transfer of labelled 3MG and of urea, both of which were used at concentrations of 1 mM l^{-1}. unless otherwise specified, were calculated from measurement of the radioactivity in the vascular effluent. Radioactivity was measured by liquid scintillation spectrophotometry using the scintillation mixture previously described (Boyd *et al.* 1975*a*); quench correction, when necessary, was performed using an external standard ratio method.

That 3MG was not metabolized during transfer was confirmed by chromatography (method of Vomhof & Tucker, 1965). The absence of transported impurities in either of the purchased ^{3}H- and ^{14}C-labelled preparations of 3MG was shown by the identity, when simultaneously measured, in the rates of transfer of both isotopes during steady state and washout experiments. [U-^{14}C]sucrose, [^{14}C]urea, 3-*O*-[^{14}C]methyl-D-glucose and 3-*O*-methyl-D-[1-^{3}H]glucose were purchased from the Radiochemical Centre, Amersham.

RESULTS

(I) *Epithelial transport of 3MG from intestinal lumen into blood*

(1) *Rate of absorption from lumen equals rate of appearance in portal outflow*

Fig. 1 shows the result of an experiment in which the absorption of 3MG from the lumen and the appearance of 3MG in the vascular effluent were measured simultaneously. The quantity absorbed is shown as A, and the quantity which is recovered in the portal vein is shown as B. The slopes of the lines A and B therefore indicate the rates of absorption and appearance respectively. Note that whereas the addition of phloretin to the vascular perfusate has no influence upon the rate of absorption from the lumen, it does increase to a small extent the rate of appearance of the sugar in the portal effluent. This finding supports the suggestion that phloretin, when present in the vascular perfusate, inhibits the entry of 3MG into compartments deep to the epithelium (Boyd, 1977). Thus in the presence of phloretin the rate of monosaccharide removal from the lumen becomes identical to the rate of appearance in the portal effluent. When phlorizin is added to the lumen later in the experiment further absorption from the lumen is quickly abolished and the rate of appearance in the vascular effluent is profoundly reduced. The finding that the rates of absorption and of appearance are identical and that phlorizin inhibits absorption and transfer indicate that the preparation is highly suited to the study of the epithelial transport of 3MG.

(2) *Flux ratio analysis of transfer*

Fig. 2 shows the result of an experiment in which the bidirectional fluxes of 3MG across the epithelium were measured simultaneously. During two separate 10 min periods (indicated by the rectangles on the abscissa) 3MG (1 mM) was introduced into both the lumen and vascular bed, for the second period phlorizin was added to the lumen. Using double isotope techniques the rate of appearance of the

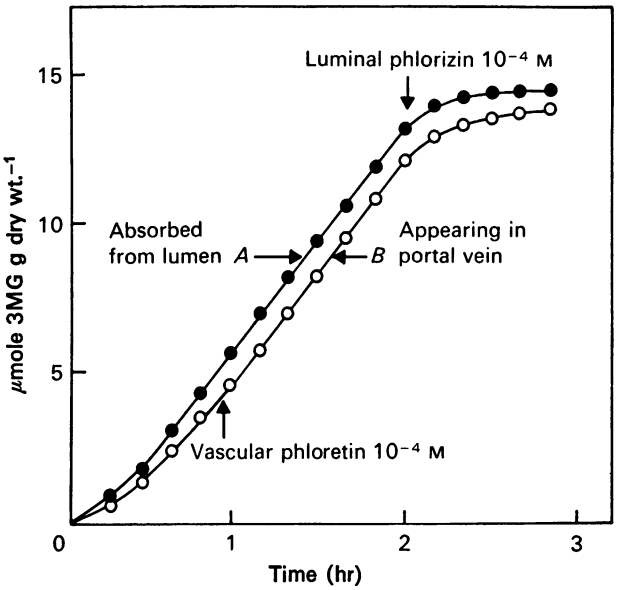

Fig. 1. The quantity of 3MG absorbed from the lumen (filled circles *A*) and appearing in the portal vein (open circles *B*). 1 mM-3MG was added to the lumen of a vascularly perfused preparation of *R. ridibunda* and was present throughout the experiment.

monosaccharide in the 'trans' compartment was measured, and is here presented on a semi-logarithmic scale. During the first phlorizin-free period, the maximal flux from lumen to vascular bed (filled circles) is some two orders of magnitude greater than the maximal flux from vascular bed to lumen (open circles); at later times the flux ratio may be even greater than this because of the differing kinetics of vascular and luminal washout (see below). The addition of phlorizin to the lumen virtually abolishes the asymmetry of the flux ratio. (Note that the total quantity of 3MG derived from the vascular bed that appears in the lumen is increased by 35%, from 61·4 to 82·8 n-mole/g dry wt. in the presence of phlorizin. This is consistent with the notion of re-uptake from the lumen of sugar derived from the vascular bed (Boyd *et al.* 1975*b*).)

(3) *Influence of rate of vascular perfusion upon the steady state transfer of 3MG*

Fig. 3 shows the relationship found in a series of frogs, between the rate of vascular perfusion and the transfer of 3MG into the portal venous effluent either with or without phlorizin present in the lumen. The rates of transfer at zero of vascular perfusion were obtained using the method of vascular stop flow described

below. It is clear that the rate of perfusion is positively related to the rate of transfer in the absence, but not in the presence, of phlorizin. In the absence of phlorizin from the lumen the rate of transfer (y) is related to the rate of vascular perfusion (x) by the expression $y = 3{\cdot}04x + 90{\cdot}4$ ($r = 0{\cdot}70$): for significance of value of slope being greater than zero, $P < 0{\cdot}05$. In the presence of phlorizin, the relationship is given by $y = 0{\cdot}23x + 2{\cdot}0$ ($r = 0{\cdot}23$; $P > 0{\cdot}5$).

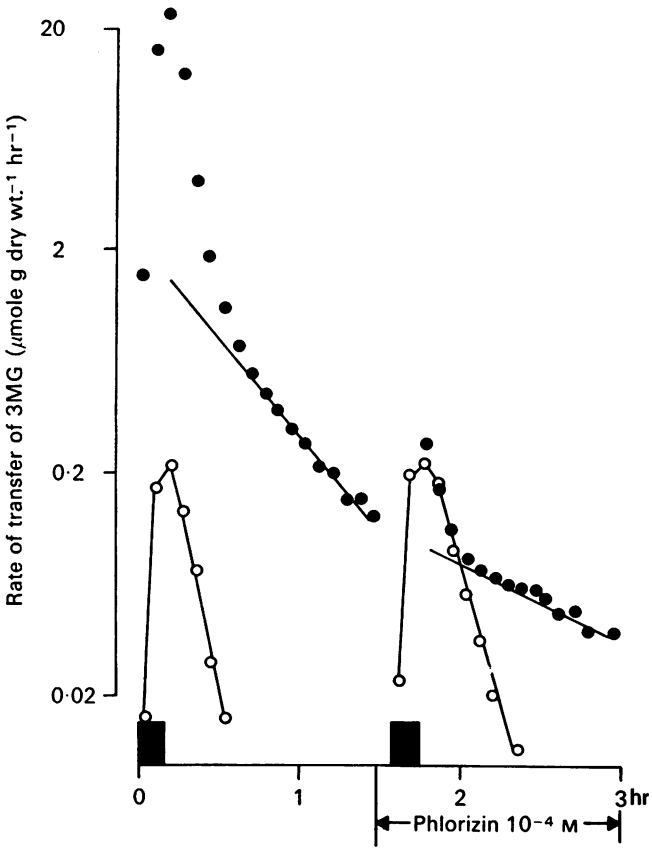

Fig. 2. The fluxes of 3MG from lumen to vascular bed (filled circles) and from vascular bed to lumen (open circles) following the addition of 1 mM-3MG to both luminal and vascular perfusates for the two periods indicated on the abscissa. Before and throughout the second period phlorizin (10^{-4} M) was added to the lumen. Semilogarithmic plot.

(II) *Perturbation experiments*

(1) *Urea*

Fig. 4 shows the washout of urea into the vascular bed, following loading from the lumen, at two differing rates of vascular perfusion. At both rates the washout is monoexponential and this is a constant finding. Associated with an increase in the rate of vascular perfusion is an increase in the rate of washout. The transfer of urea from the lumen to the vascular bed is also greater with a faster rate of vascular flow as may be seen from the peak rates of transfer. This is also found under steady-state conditions (Boyd, 1976), so that urea transfer may be described as being 'flow dependent'.

(2) *3MG*

Unlike the case for urea, the washout of 3MG following loading from the lumen is more complicated than a single exponential process. The rate of vascular appearance during washout can, however, be described satisfactorily by the sum of two exponentials (Figs. 5, 7), with rate constants K_1 ('fast') and K_2 ('slow'). The rate

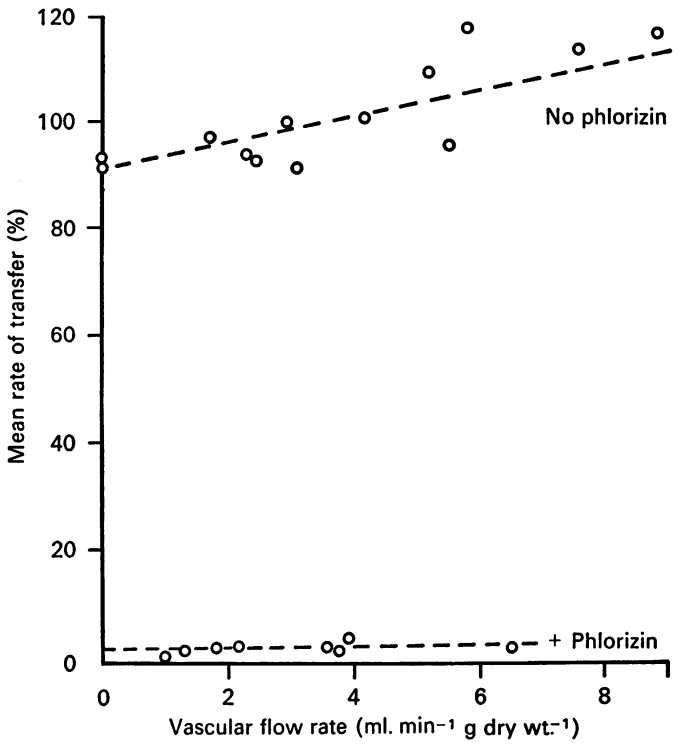

Fig. 3. The influence of the rate of vascular perfusion upon the steady state rate of transfer of 3MG from the lumen (1 mM) into the vascular bed either with (lower line) or without (upper line) phlorizin (5×10^{-5} M) present in the lumen. Na was present in the lumen in both groups of experiments. The results are for a series of *R. ridibunda* and have been normalized; thus at a vascular flow rate of 4 ml. min^{-1} g dry $wt.^{-1}$ (= 100% mean rate of transfer) the rate of transfer was $6{\cdot}89 \pm 0{\cdot}88$ (5) μmole g dry $wt.^{-1}$ hr^{-1}.

of washout into the vascular bed of 3MG is characterized by the sensitivity of the rate constants K_1 and K_2 to the rate of vascular perfusion (Fig. 6). Whereas K_1 is clearly related to the vascular flow rate K_2 appears to be largely independent of the rate of perfusion. Pooling the results from a series of animals (Fig. 6) reveals that on extrapolation to zero rate of vascular perfusion K_1 and K_2 appear to become identical, as if in the absence of vascular perfusion the tissue would drain slowly from a single pool at a rate characterized by the slower rate constant K_2.

The influence of phloretin, added to the vascular perfusate, upon the washout of previously loaded 3MG is shown in Fig. 5. In the presence of phloretin 3MG

washout more resembles a monoexponential process than is found during the markedly biphasic washout following the control pulse. It is as if the filling from the lumen of the more slowly draining pool is decreased by the presence of phloretin in the vascular bed.

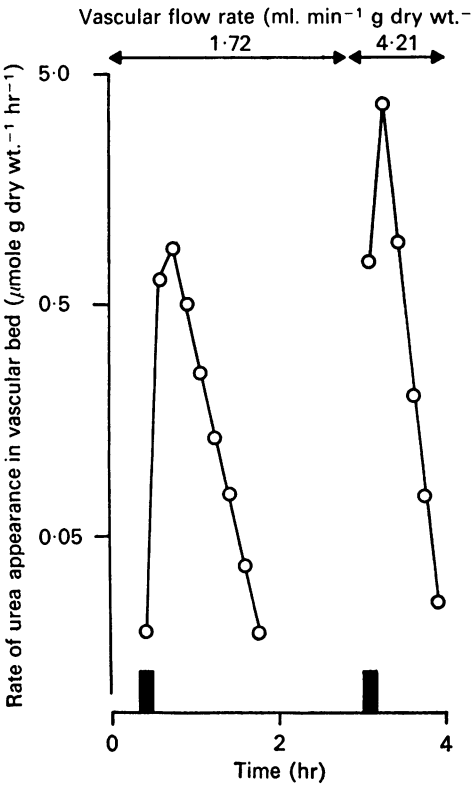

Fig. 4

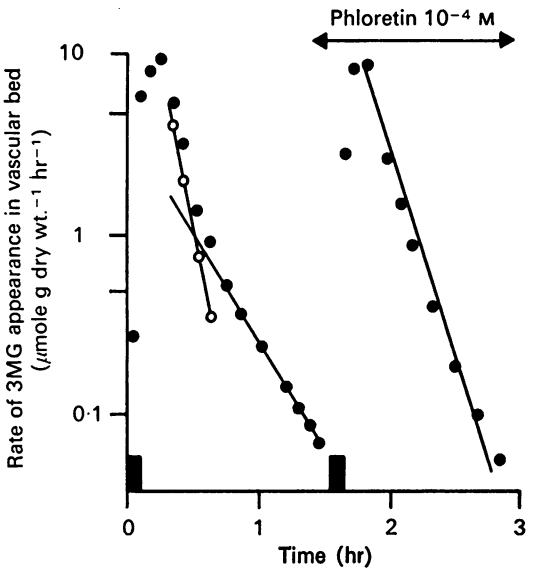

Fig. 5

Fig. 4. The rate of washout into the vascular bed of urea following the addition of 1 mM urea to the lumen for the two periods indicated on the abscissa, first at a low (1·72 ml. min^{-1} g dry wt.$^{-1}$) and subsequently at a faster (4·21 ml. min^{-1} g dry wt.$^{-1}$) rate of vascular perfusion. Semilogarithmic plot. *R. pipiens*.

Fig. 5. The rate of washout of 3MG into vascular bed following two periods of loading from the lumen (indicated on the abscissa) with 1 mM monosaccharide. During the second period, phloretin (10^{-4} M) was added to the vascular perfusate. Semilogarithmic plot. Open circles represent values derived after subtraction of the contribution of the more slowly draining pool, i.e. after the curve has been 'stripped'.

If phlorizin is added to the lumen at the start of the period of washout, the rate constant K_1 for 3MG unloading into the vascular bed is increased (see Fig. 7; Table 1). Associated with this increase in K_1 is a decrease in the total quantity of 3MG appearing in the portal effluent during the washout. Thus in the presence of phlorizin there is a decrease in the total quantity of 3MG that is recovered from the tissue in the portal effluent. With phlorizin in the lumen the mean size of the pool(s) that unload into the portal effluent in the presence of phlorizin is reduced to 59% of that in its absence (Table 2). The data in this Table also show that, in

TABLE 1. Washout into vascular bed of 3MG absorbed from lumen (1 mM). Influence of addition to lumen of phlorizin at start of washout upon the rate constants K_1 and K_2

Condition in lumen during washout	K_1 (% control)	K_2 (% control)
Control (no addition)	100	100
Phlorizin (5×10^{-5} M)	181 ± 13 (5)	104 ± 6 (5)

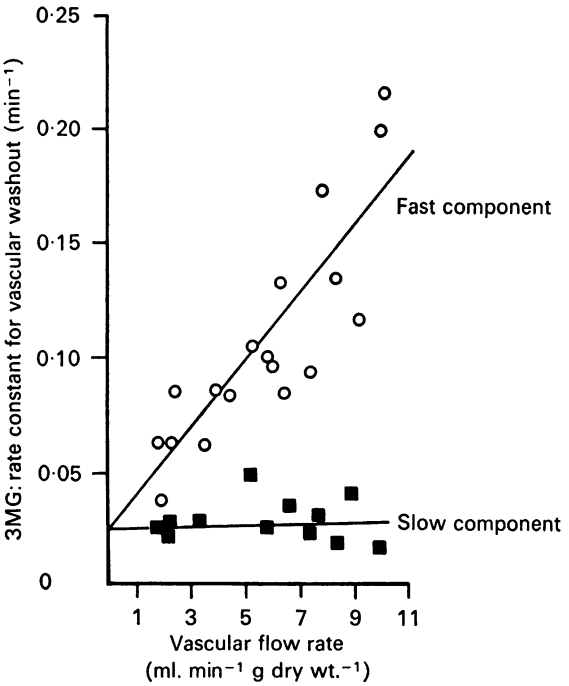

Fig. 6. The relationship between the rate of vascular flow (abscissa) and the rate constants describing the washout of 3MG, absorbed from the lumen, into the vascular bed (ordinate). The open circles represent the rate constants for the faster draining pool; the squares the rate constants for the more slowly draining pool. Note the similar intercept on the ordinate at zero rate of vascular flow. Data derived from a series of experiments on *R. pipiens*.

TABLE 2. The size of the pool of 3MG found in the vascularly perfused intestine upon loading (1 mM 3MG in the lumen) and upon unloading with and without the addition of phlorizin to the lumen during washout. Results are expressed as % of the loading pool, the mean size of which was $0{\cdot}91 \pm 0{\cdot}32$ (11) μmole g dry wt.$^{-1}$

Loading pool	Unloading pool	Unloading pool + phlorizin 5×10^{-5} M
100	182 ± 4 (6)	108 ± 6 (4)

the absence of phlorizin during the washout, the size of the pool(s) that have to be loaded up before the steady state of absorption is achieved ('loading pool') is less than that of the 'unloading pool'. This 'up–down asymmetry' exhibited by the epithelium is virtually abolished by the addition of phlorizin to the lumen during the unloading.

(III) *Vascular stop flow*

The rate of appearance of 3MG and urea in the portal effluent following a 30 min period of vascular stoppage is shown in Fig. 8. This experiment clearly indicates the consistent findings seen following stoppage. Transient increases in the rate of appearance, over and above the steady state rate of transfer, are seen. The transient

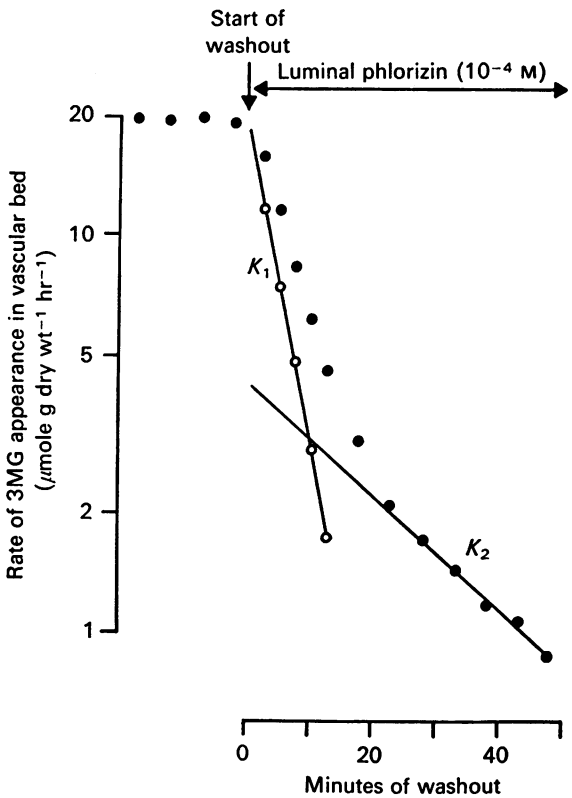

Fig. 7. The kinetics of the washout of 3MG into the vascular bed following the addition of phlorizin to the lumen at the start of the washout. K_1 and K_2 represent the rate constants for the fast (open circles 'stripped' curve) and slow phases of the washout. The vascular flow rate was 5·1 ml. min^{-1} g dry wt.$^{-1}$; and the concentration of 3MG in the lumen during the loading phase was 1 mM. Semilogarithmic plot.

for urea is, however, markedly less than for 3MG, in particular the peak is lower and the transient lasts for a shorter period. In this experiment, after the vascular pump was restarted following stoppage, there is an immediate fall in the observed rate of 3MG appearance. This phase, which is pronounced only if the 'vascular pump' is switched off (see Methods), lasts for approximately the first 2 min and is followed by a large transient increase in the appearance rate. This is not the case for urea, when the initial fall at the start of washout is not seen.

Integration of the area under the transient peak observed in experiments of this sort allows estimation of the extra quantity of solute that is recovered in the vascular bed as a consequence of stoppage. In this way accumulation of 3MG, but

not of urea, within the total tissue water is clearly demonstrable. The extent of such accumulation of the monosaccharide within the tissue is found to depend upon a number of factors, for example, upon the presence or absence of Na ions in the intestinal lumen (Fig. 9), and upon the duration of the stoppage. The trapping of urea within the tissue is only influenced to a small extent by the presence of Na ions; and the duration of stoppage does not influence the quantity of urea trapped once this solute is in equilibrium with the tissue. However, for 3MG the extra

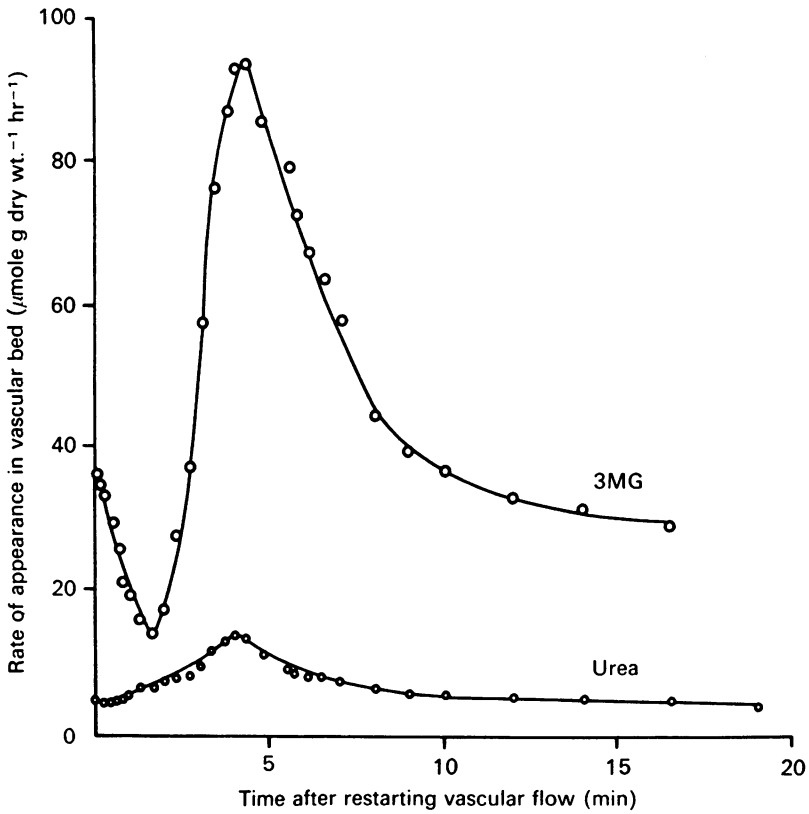

Fig. 8. The rate of appearance in vascular effluent of 3MG (large circles) and of urea (small circles) immediately following the restart of vascular flow after a 30 min period of vascular stop. Urea and 3MG (each 1 mM) were both present in the lumen throughout the experiment.

quantity recovered is not linearly related to the duration of stoppage. Thus (Fig. 10) as the period of stoppage is prolonged the rate of transport during stoppage, as measured by the recovery of 3MG upon resumption of flow, is decreased (Table 3).

The combination of the experimental manoeuvres of periods of vascular stoppage and of changes in the steady state rate of vascular perfusion upon a single preparation allows the concentration of 3MG in the tissue to be computed at a number of differing rates of vascular flow. The tissue concentrations for both 3MG and urea, with and without Na present in the luminal perfusate, are shown in Fig. 11. Note that the concentrations observed at zero vascular flow are those for a 30 min

stoppage; they will obviously be dependent upon the period of vascular stop (Fig. 10). The tissue concentration of 3MG is markedly influenced by the presence or absence of Na; this effect is not seen for urea. This Figure shows clearly the conditions required for tissue accumulation to be seen: they are that the solute be actively transported (3MG but not urea); that Na ions be present in the luminal perfusate,

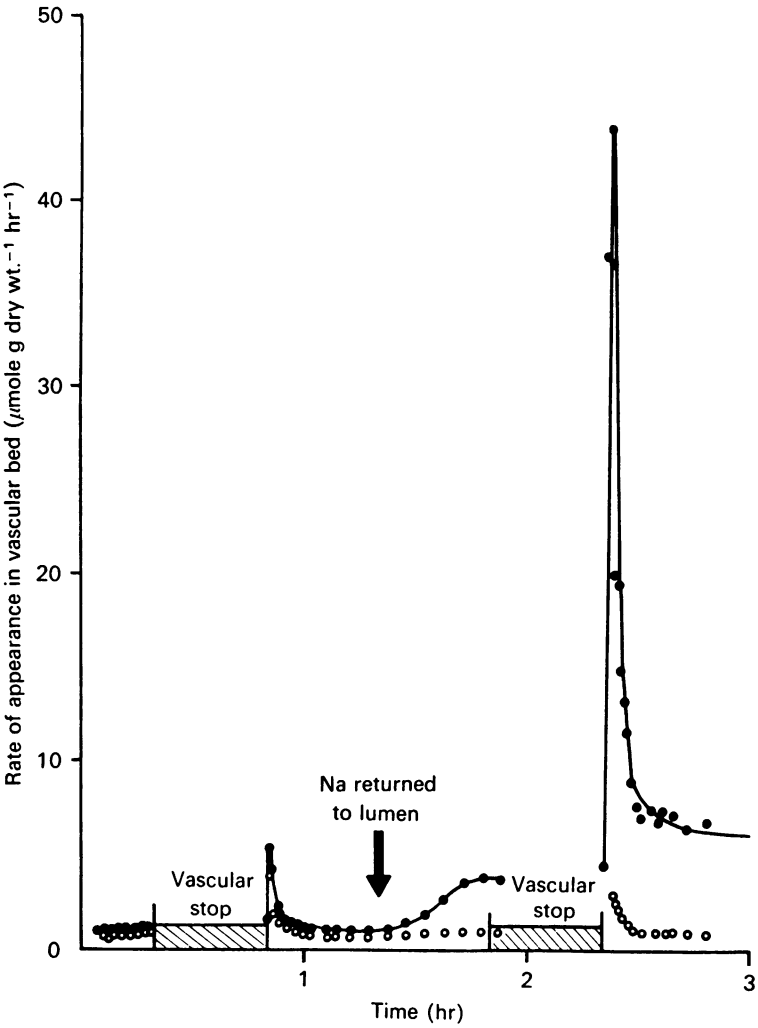

Fig. 9. The rate of appearance in the vascular bed of 3MG (closed circles) and of urea (open circles) following the addition of both compounds to the lumen (1 mM), initially in the absence of Na from the lumen, subsequently with Na present in the lumen. In each case vascular flow was interrupted for 30 min.

and that vascular flow through the epithelial bed be absent. The reciprocal relationship between the tissue concentration and the vascular flow rate is found for both substrates, with and without Na in the lumen, but the relationship is clearly more marked for 3MG in the presence of Na.

Following stoppage, the rate of washout of the solute trapped in the tissue is

determined by the rate of perfusion. Thus Fig. 12 shows the vascular appearance rate of 3MG following three identical 30 min periods of stoppage performed at differing steady state rates of vascular flow. Providing that there is an adequately fast rate of perfusion following stoppage, the washout of the extra 3MG trapped in the tissue cannot be described by a single exponential. Typically, a result such as that shown in Fig. 13 is seen with a very fast rate of washout (characterized by rate constant K_A) being followed by a slower process described by rate constant K_B. Interestingly the trapped urea washout following stoppage shows such double exponential kinetics. Although analogous to the double exponential washout seen for 3MG in perturbation experiments it should be noted that the time scale of the processes is very different.

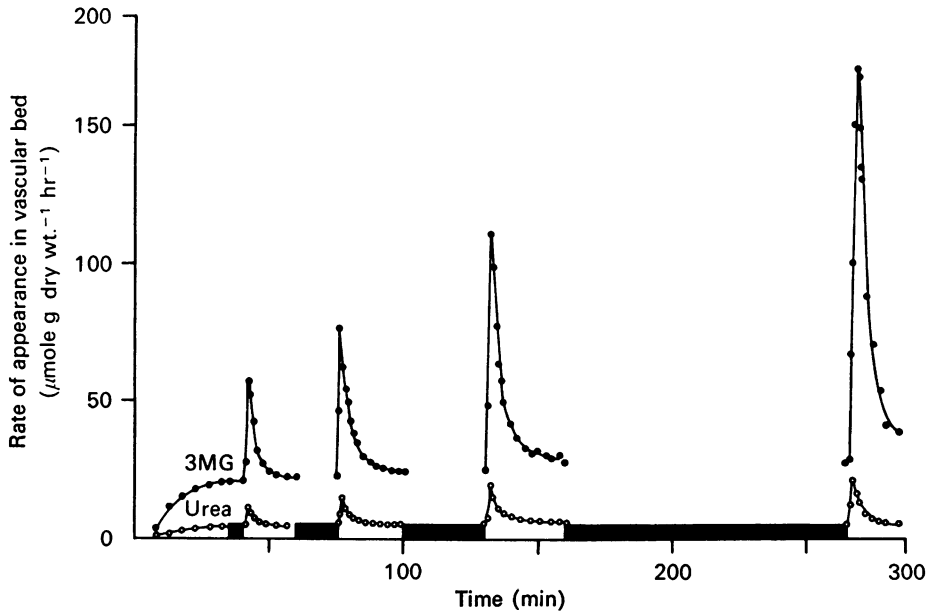

Fig. 10. The rates of appearance of 3MG (filled circles) and of urea (open circles) in the vascular bed following four periods of vascular stop (indicated by the filled rectangles on the abscissa) of respectively 5, 15, 30 and 90 min duration.

TABLE 3. The effects of four differing lengths of vascular stoppage upon the tissue pool of 3MG. The results of a single representative experiment are shown. The pool size in the steady state (i.e. with 0 min of stoppage) is that derived from the loading pool. The predicted pool is derived by the addition to this of the product of the steady state rate of transfer and the length of stoppage. Note the increasing loss of 3MG from the tissue (i.e. the decreased 'recovery') as the period of vascular stoppage is prolonged. The luminal concentration of 3MG was 1 mM throughout the experiment

Length of stop (min)	Observed pool size (μmole g dry wt.$^{-1}$)	Predicted pool size (μmole g dry wt.$^{-1}$)	'Recovery' (observed/predicted) (%)
0	2·0	2·0	100
5	3·8	3·83	99
15	6·0	7·5	80
30	10·0	13·0	75
90	16·0	35·0	46

DISCUSSION

Influence of vascular flow on transfer of 3MG from lumen to blood

The rate of 3MG absorption from the lumen and the rate of appearance of the sugar in the portal venous effluent are found to be nearly, but not quite, equal (Fig. 1). When phloretin is added to the vascular bed the rates of absorption and transfer do become identical presumably because phloretin blocks access of the

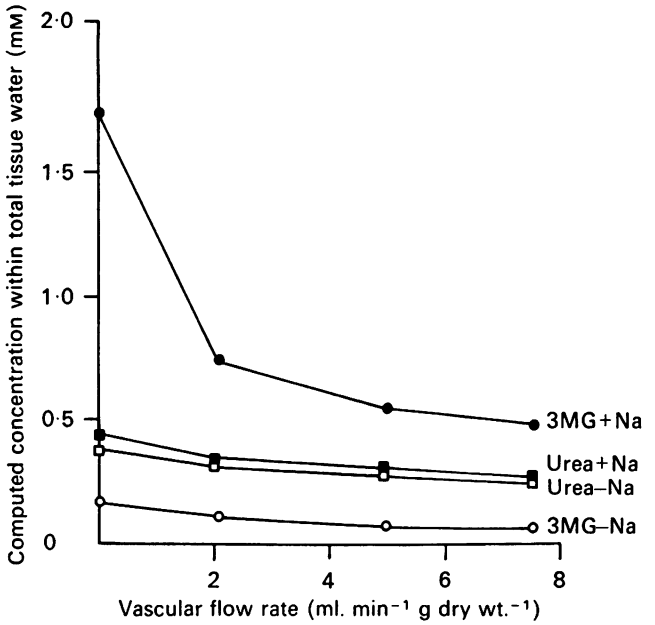

Fig. 11. Result of a typical individual experiment showing the influence of the rate of vascular perfusion upon the quantity of urea and of 3MG present within the whole intestine; these results were obtained initially with Na absent from (choline substitution) and subsequently with Na present in both the vascular and luminal perfusates. The solute concentration within the tissue water was computed from the size of the loading pool (Boyd *et al.* 1975*a*) and from the transients in the rate of solute appearance observed upon changing (both increasing and decreasing) the vascular flow rate, the period of vascular stop was 30 min; a longer period would increase, and a shorter period decrease the concentrations indicated by the points on the ordinate. The concentration of both solutes in the lumen was 1 mM.

monosaccharide to some pool (such as muscle) deep to the epithelium. It is of considerable interest that phloretin added to the vascular perfusate does not decrease the rate of 3MG transfer, since Kimmich & Randles (1975) have shown that phloretin does decrease the entry of sugars into isolated intestinal epithelial cells across the basolateral membrane. This discrepancy might indicate that, *in vivo*, sugar entry into and exit from the epithelium across the basolateral membrane are not by identical processes.

In the steady state, with Na present in the intestinal lumen, the rate of vascular perfusion does not have a striking effect on the transfer of 3MG. Nevertheless there is a significant positive correlation between the rate of vascular flow and the rate

of sugar transfer (Fig. 3), indicating that *in vivo* the work performed by the heart in perfusing the subepithelial capillary bed does contribute to the transfer of solute across the epithelium. Sanderson (1977) has recently shown similar effects on amino acid transfer. The increased flux of 3MG at faster rates of vascular perfusion must involve membrane transport mechanisms (rather than merely diffusion) since the increase in the flux from the lumen to the vascular bed at faster rates of vascular perfusion is not found in the presence of phlorizin in the lumen.

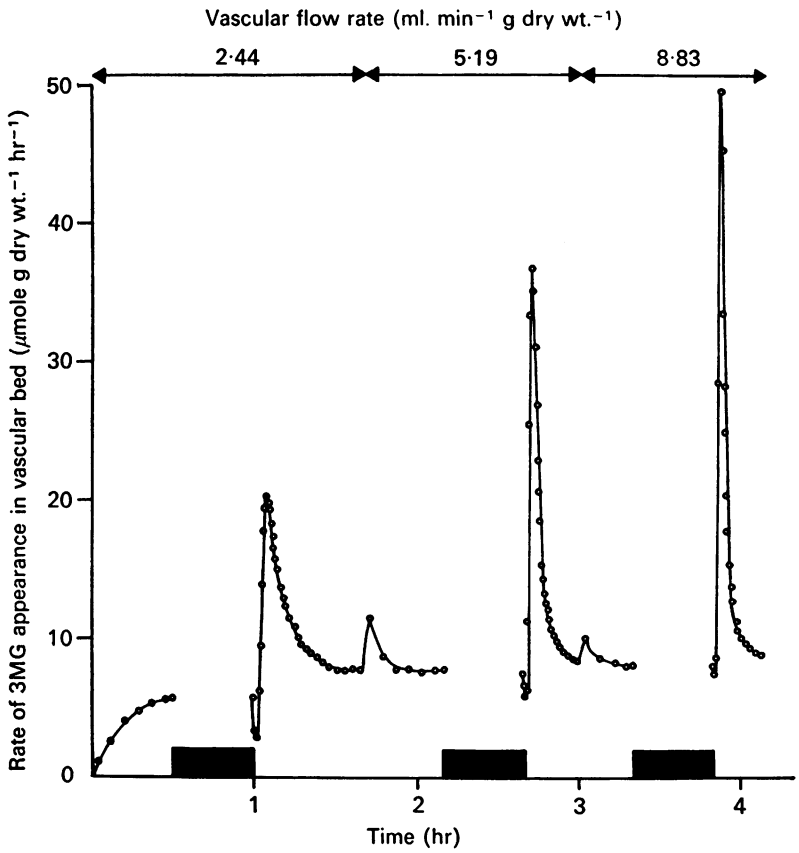

Fig. 12. The effects of changing the rate of vascular perfusion (indicated by the arrows at the top of the figure) upon the rate of appearance of 3MG in the vascular bed following vascular stop. The sugar (1 mM) was perfused through the intestinal lumen throughout the experiment. Thirty-minute periods of vascular stop (indicated by the rectangles on the abscissa) were performed at each of the three rates of vascular perfusion. Note that an increase in the rate of perfusion is clearly accompanied by an increase in the rate of vascular appearance of monosaccharide trapped in the tissue during vascular stoppage. In contrast, the rate of perfusion has only a small influence upon the steady state rate of transfer (see also Fig. 3). Note also the transient increase in the 3MG rate of appearance that accompanies each abrupt increase in the rate of vascular flow.

Perturbation experiments

Double exponential washout of 3MG into the effluent appearing from the portal vein

Unlike urea, which drains into the vascular bed at a rate that may be characterized by a single exponential, the washout of 3MG into the venous effluent following loading from the lumen is more complex. The washout of the sugar may be satisfactorily described by the sum of two exponentials but this does not mean that there are necessarily only two compartments involved. Even if this assumption is made the relationship between the compartments, whether catenulate or in parallel, is uncertain. However, one clear distinction between the rate constants

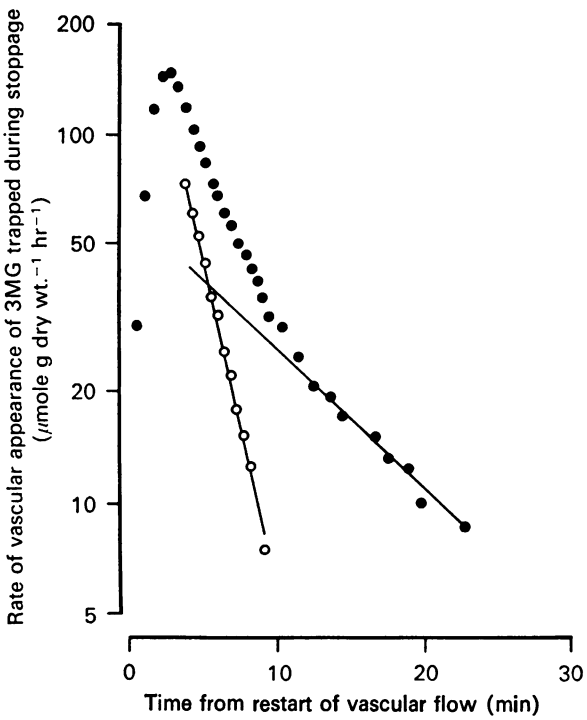

Fig. 13. The rate of appearance in the portal vein immediately following the restart of vascular perfusion, of the extra 3MG that has been entrained in the tissue following a 30 min period of vascular stop. 3MG (1 mM) was present in the lumen throughout the experiment. Note the double exponential washout of the 3MG that has been trapped within the tissue during the period of vascular stop. Semilogarithmic plot. Open circles represent values derived after subtraction of the contribution of the more slowly draining pool.

K_1 and K_2, characterizing the faster and slower phases of the washout, is found: the former is relatively sensitive and the latter insensitive to the rate of vascular perfusion (Fig. 6). The finding that, on extrapolating to zero vascular flow, the rate constants K_1 and K_2 become identical suggests that the washout of transported substances from the epithelium in preparations of intestine that lack a blood supply is limited by the slow rate constant K_2. Such an explanation is in keeping with the

high tissue concentrations of solute observed during transport with *in vitro* but not with *in vivo* preparations.

Possible compartments from which the 3MG may drain into the vascular bed include the epithelium, the tissues deep to the epithelium and the lumen that is adjacent to the brush border. There is evidence that all three may be involved.

Thus, the finding (Fig. 5) that phloretin, when added to the vascular bed, distorts the kinetics of 3MG washout into the portal effluent is in keeping with the suggestion that in the absence of phloretin the tissues deep to the epithelium may become loaded with the sugar, initially added to the lumen, and hence contribute to the observed pool of 3MG that drains into the vascular bed during washout.

Similarly, the finding that the addition of phlorizin to the lumen at the start of the washout increases the rate constant K_1 suggests that the luminal contents that are adjacent to the brush border are involved. The simplest explanation is that the tissue pool of 3MG, as observed in such washout experiments, includes solute trapped in a boundary layer within the intestinal lumen ('luminal unstirred layer'). On the basis of this hypothesis the addition of phlorizin during washout will abolish brush border entry and the subsequent appearance in the portal effluent of 3MG present within this boundary layer. The net effect will be to decrease the observed size of the tissue pool (Table 2) and to increase K_1 (Table 1) as a consequence of the reduction in the mean path length (λ) from the pool to the vascular bed. The morphology of the intestinal surface, in particular the presence of spaces between the corrugations of the epithelial surface that in the frog are analogous to the mammalian inter-villus spaces (Wilczynska, 1975), supports the hypothesis that the intestinal lumen may, from a physiological standpoint, be divisable into a dead space (the bulk phase) and a boundary layer.

TABLE 4. The values of the effective diffusion coefficient D' for urea and for 3MG calculated, for path lengths (λ) of 100 and 200 μm respectively, using the washout rate constant, K_1, found at a vascular flow rate of 5 ml. min^{-1} g dry wt.$^{-1}$ (see Figs. 4 and 6). D is the value of the diffusion coefficients of the solutes in free solution at 19 °C (Weast, 1970). See text.

	λ = 100 μm		λ = 200 μm	
	Urea	3MG	Urea	3MG
K (sec^{-1})	$2{\cdot}52 \times 10^{-3}$	$1{\cdot}33 \times 10^{-3}$	$2{\cdot}52 \times 10^{-3}$	$1{\cdot}33 \times 10^{-3}$
D' (cm^2 sec^{-1})	$1{\cdot}02 \times 10^{-7}$	$0{\cdot}54 \times 10^{-7}$	$4{\cdot}09 \times 10^{-7}$	$2{\cdot}16 \times 10^{-7}$
D (cm^2 sec^{-1})	$1{\cdot}20 \times 10^{-5}$	$0{\cdot}60 \times 10^{-5}$	$1{\cdot}20 \times 10^{-5}$	$0{\cdot}60 \times 10^{-5}$
$(D'/D) \times 10^2$	0·85	0·90	3·41	3·60

If we now assume from these arguments that the fast phase of 3MG washout into the vascular bed is from the epithelium itself, and also that diffusion from the epithelium is rate limiting to the transfer into the vascular bed we can relate the observed rate constant (K_1) for the washout to the diffusion path length (λ) and to the effective diffusion coefficient (D') by the relationship, derived by Hill (1928) (see Keynes, 1954) for washout from one face of a plane sheet of tissue, the other face being impermeable,

$$\lambda^2 = \frac{\pi^2 D'}{4K}.$$

(We assume that, following loading from the lumen, the perfused intestine behaves as a plane sheet impermeable to 3MG backflux into the lumen and that the last two thirds of the washout are considered.) Since, for 3MG, K_1 is a function of the rate of vascular perfusion (Fig. 6), we can see that an increase in the rate of perfusion may result either, for a given value of D' in a decrease in path length λ; or, for a given value of λ in an increase in the effective diffusion coefficient. Values of D' are derived in Table 4 both for urea and for 3MG, using the values of K found at a vascular flow rate similar to that found *in vivo* (Prichard, 1966), assuming the diffusion path length from epithelium to vascular bed to be either 100 or 200 μm. Using the latter value we find the value of the effective diffusion coefficient to be approximately 4% of the free solution value. Silverman (1976) has recently used a value of 10% of the coefficient in free solution to explain the observed rate of transepithelial flux of glucose in the intact kidney.

Evidence for recycling across the epithelium

Fig. 2 reveals that the total quantity of 3MG, initially added to the vascular bed, that is recovered in the luminal effluent is increased when phlorizin is added to the lumen. This implies that the 'unidirectional' fluxes as usually measured between blood and luminal bulk phase may be underestimates of the real values because of re-uptake of solute at the brush border. Such recycling may occur during absorption *in vivo*, as indicated by the influence of phlorizin upon the washout pool size and rate constants discussed above, and would account for the presence of a layer in the lumen adjacent to the epithelium in which solute or ion activity might differ from that in the luminal bulk phase (White, 1976).

Vascular stop flow

Our observation of the relationship between the rate of vascular flow and the accumulation of actively transported solutes in the tissue is of significance with respect to findings with classical *in vitro* preparations. It is clear that the accumulation of 3MG, but not of urea, is demonstrable in the preparation of frog small intestine by the simple expedient of stopping vascular perfusion; it is also clear that accumulation of this sugar within the whole tissue is not found during vascular perfusion. This latter finding does not, of course, mean that we can exclude the possibility that during transfer there is accumulation within a small compartment of the tissue. We have shown that using vascular stoppage, the same intestine may be easily and reversibly switched from simulating the behaviour of the intestine *in vivo* to simulating the intestine *in vitro* merely by altering the rate of vascular perfusion. The high tissue concentrations seen during stoppage must be borne in mind when interpreting findings made using *in vitro* preparations. In particular the high concentration in the tissue in *vitro* will be expected to result in a much greater efflux of solute from the epithelium back into the lumen ('back flux'); this raises questions both as to the predicted solute concentration within the luminal boundary layer in *in vitro* preparations, and as to the energetics of such amplified solute recycling. Such exaggerated solute back flux is clearly shown to increase with prolonging the period of stoppage (Fig. 10, Table 3). This sort of finding must

help to explain why the net rates of transfer *in vitro* are only a fraction of those seen *in vivo*.

When considering the transport properties of the vascularly perfused preparation it is important to realize that it is not solely the epithelium that is perfused. For example, the vascular bed of the intestinal smooth muscle, in parallel to that of the epithelium, will also be perfused by fluid introduced into the mesenteric artery. Such tissues deep to the epithelium (with respect to the intestinal lumen) will act as capacitors. Thus during vascular stoppage solute will diffuse into them at a rate dependent on the permeability properties of the tissue with respect to the solute molecules. Upon resumption of vascular flow the solute will be recovered from both the epithelium and from such deeper tissues. The initial pattern of the rate of appearance of urea and of 3MG that is seen following complete stoppage may depend upon the drainage of such a capacitance preceding epithelial perfusion. However, perfusion of the epithelium itself seems to be compromised at the very low rates of vascular flow used in the routine stoppage procedure, probably because of the shunting of the perfusate through such deeper capacitance tissues. The term vascular stop flow therefore remains appropriate.

Following stoppage the washout of trapped 3MG is found to be well described by a double exponential. In contrast to that seen in perturbation experiments this process occurs over a much shorter period of time. A plausible explanation for the relationship between these rate constants is that K_B (the slow rate constant of vascular stop flow experiments) reflects the same processes as does the fast rate constant K_1 of the perturbation experiments. The absence in post stoppage experiments of a rate constant equivalent to the slow rate constant K_2 of perturbation experiments is almost certainly a reflexion of the technical problem of measuring such a very slow rate of washout over and above the steady state rate of transfer.

'Up–down asymmetry'

'Up–down asymmetry', the finding that the unloading pool appears larger than the loading pool, is determined apparently by events associated with the brush border as indicated by the loss of such asymmetry upon the addition of phlorizin to the lumen during unloading. The mechanism underlying this phenomenon, which in effect makes the luminal boundary layer appear thicker during unloading than during loading awaits a decisive explanation. The binding of solute to the glycocalyx in such a way that it can form a 'store' to be subsequently transported might provide one sort of explanation. It is interesting that the phenomenon of up–down asymmetry is not confined to monosaccharides in the intestine; thus it has been found in the frog skin for Na (Fuchs, Gebhardt & Lindeman, 1972) and in skeletal muscle for K (Hodgkin & Horowicz, 1960).

Conclusions

We conclude first, that, for the actively transported 3MG the extent of accumulation within the tissue is closely related to the rate at which the substance can be cleared from the tissue and that this clearance is itself a function of the rate of vascular perfusion. Other factors, for example the presence or absence of Na in the intestinal lumen, the exit permeability from the epithelial cells, must also

determine the extent of accumulation found during a given rate of perfusion. These factors will be considered elsewhere.

Secondly, there is significant recycling of 3MG between the epithelium and the fluid in the lumen that is adjacent to the epithelium. This finding raises questions as to whether this fluid can be regarded as a compartment of the intestinal epithelium and whether the bulk contents of the intestinal lumen occupy a physiological dead space.

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